(11) EP 0 911 396 A3

(12)

EUROPEAN PATENT APPLICATION

- (88) Date of publication A3: 06.05.1999 Bulletin 1999/18
- (43) Date of publication A2: 28.04.1999 Bulletin 1999/17
- (21) Application number: 98122014.8
- (22) Date of filing: 02.12.1996

- (51) Int CL⁶: **C12N 15/10**, C12N 15/64, C12Q 1/68 // C07K14/435, C07K14/545, C12N9/86, C12N9/38, C12N15/52
- (84) Designated Contracting States:

 AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC

 NL PT SE
- (30) Priority: 30.11.1995 US 564955 25.03.1996 US 621859
- (62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC: 96940934.1 / 0 876 509
- (71) Applicant: Maxygen, Inc. Santa Clara, CA 95051 (US)

- (72) Inventors:
 - Stemmer, Willem P.C. Los Gatos, CA 95030 (US)
 - Crameri, Andreas
 Mountain View, CA 94040 (US)
- (74) Representative: Irvine, Jonquil Claire
 J.A. KEMP & CO.
 14 South Square
 Gray's inn
 London WC1R 5LX (GB)
- (54) Methods for generating polynucleotides having desired characteristics by iterative selective and recombination
- (57) The present invention relates to a method for evolving a polynucleotide encoding a plurality of genes, e.g. multiple genes forming a multicomponent pathway. The method involves shuffling of polynucleotides by conducting a polynucleotide amplification process on overlapping segments of a population of variants of a polynucleotide encoding a plurality of genes under conditions whereby one segment serves as a template for extension of another segment to generate a population of recombinant polynucleotides. This population is screened for a recombinant polynucleotide encoding a plurality of genes having a desired property.

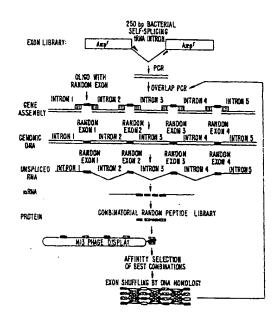


FIG. 20.



EUROPEAN SEARCH REPORT

Application Number EP 98 12 2014

	Citation of document with indication, where appropriate. Relevant			CLASSIFICATION OF TH		
Category	of relevant pass		to claim	APPLICATION (Int.CI.6)		
Υ	WO 95 22625 A (AFFY WILLEM P C (US); CR 24 August 1995 * the whole documen	1-20	C12N15/10 C12N15/64 C12Q1/68 //C07K14/435,			
	STEMMER W: "DNA sh fragmentatio and re recombination for m PROCEEDINGS OF THE SCIENCES OF USA, vol. 91, October 19 XP002087463 * the whole documen	assembly: In vitro olecular evolution" NATIONAL ACADEMY OF 94, pages 10747-10751,	1-20	C07K14/545, C12N9/86, C12N9/38, C12N15/52		
	STEMMER W P C: "Rap protein in vitro by NATURE, vol. 370, 4 August 1 XP002082182 * the whole document	DNA shuffling" 1994, pages 389-391,	1-20			
	W.P.C. STEMMER: "Se space" NATURE BIOTECHNOLOGY vol. 13, June 1995, XP002095510 NATURE PUBL. CO.,NEW * the whole document	, pages 549-553, I YORK, US	1-20	TECHNICAL FIELDS SEARCHED (Int.Cl.8) C12N C12Q		
	US 5 279 952 A (WU KUN C) 18 January 1994 * the whole document *		1-20			
US 5 223 408 A (GOE 29 June 1993 * the whole documen		DDEL DAVID V ET AL)	1-20			
	The present search report has b	een drawn up for all claims				
	Place of search	Date of completion of the sear h		Examiner		
THE HAGUE		4 March 1999	Hor	Hornig, H		
X : partic Y : partic docui A : techr	TEGORY OF CITED DOCUMENTS cutarly relevant if taken alone cutarly relevant if combined with another ment of the same category sological background written disclosure	To theory or since Elearner patent of after the tring of Disdocument of Lindocument of the 8, member of the	c rumant but publiste Late Late Application for their reasons	hished on, or		



EUROPEAN SEARCH REPORT

Application Number EP 98 12 2014

Category	Citation of document with of relevant pas	indication, where appropriate, sages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	genes without the u enzymes: gene splic extension" GENE.	eing by overlap es 61-68, XP002090392	1-20	
			1-20	
	WO 91 07506 A (US G * the whole documen	OVERNMENT) 30 May 1991 t *	1-20	
	POSTTRANSLATIONAL A FLUORESCENT PROTEIN	NATIONAL ACADEMY OF 1994, pages 74454	1-20	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
	A. CRAMERI ET AL.: fluorescent protein using DNA shuffling NATURE GENETICS, vol. 14, March 1996 XP002095449 NATURE PUBLISHING C * the whole documen	by molecular evolution , pages 315-319, O., NEW YORK, US	1-16	
	The present search report has	been drawn up for all claims		
	Place of search	Date of completion of the search		Examiner
	THE HAGUE	4 March 1999	Hor	nig, H
X : parti Y : parti docu A : techi O : non-	ATEGORY OF CITED DOCUMENTS cularly relevant if taken atone cularly relevant if combined with anot ment of the same category notogical background with disclosure mediate document	E : earlier patent do after the filing da	cument, out publis te in the application or other reasons	shed on, of

PO ECISIO 1803 GR PO CO



EUROPEAN SEARCH REPORT

Application Number EP 98 12 2014

ategory	Citation of document with indicate	ion, where appropriate.	Relevant	CLASSIFICATION OF THE
ategory.	of relevant passages		to claim	APPLICATION (Int.Cl.6)
-	WO 97 35966 A (MAXYGEN JEREMY (US); STEMMER W 2 October 1997 * examples I-IV. *		1-16	
T .	CRAMERI A ET AL: "Mole an arsenate detoxificat shuffling" NATURE BIOTECHNOLOGY, vol. 15, May 1997, page XP002082183 * the whole document *	ion pathway y DNA	1-16	
				TECHNICAL FIELDS
				SEARCHED (Int.Cl.6)
	The present search report has been o	Date of completion of the search	1	Examiner
	Place of search TUC MACHE	4 March 1999	Hor	nig, H
X : part Y : part doc A : tech	THE HAGUE ATEGORY OF CITED DOCUMENTS icularly relevant if taken alone icutarly relevant if combined with another ument of the same category mological backgroundwritten disclosure	T : theory or princ E : earlier patent after the filing D : document cite L : document cite	iple underlying the i document, but publi- date d in the application d for other reasons	nvention shed on, or

EPO FORM 1503 03.82 (P04C01)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO.

EP 98 12 2014

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

04-03-1999

Patent docume cited in search re		Publication date	1	Patent family member(s)	Publication date
WO 9522625	А	24-08-1995	US AU CA CN EP JP US	5605793 A 2971495 A 2182393 A 1145641 A 0752008 A 10500561 T 5811238 A 5837458 A	25-02-199 04-09-199 24-08-199 19-03-199 08-01-199 20-01-1998 22-09-1998
US 5279952	 A	18-01-1994	NONE		
US 5223408	Α	29-06-1993	· US	5736135 A	07-04-1998
WO 9107506	Α	30-05-1991	AU	6886991 A	13-06-1991
wo 9735966	Α	02-10-1997	US AU AU WO AU CA EP WO	5837458 A 2337797 A 2542697 A 9735957 A 1087397 A 2239099 A 0876509 A 9720078 A	17-11-1998 17-10-1997 17-10-1997 02-10-1998 19-06-1997 05-06-1997 11-11-1998 05-06-1997

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

